

# VOLATILE GROWTH INHIBITORS PRODUCED BY *ECUALYPTUS GLOBULUS*

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The possibility that plants of some species gain advantage in natural competition by the excretion of substances (exocrines or ectocrines) inhibitory to the growth of their potential neighbors is under investigation in a number of laboratories. Among these investigations, the studies by Muller and his co-workers on volatile substances produced by aromatic shrubs have aroused considerable interest. Working particularly with *Salvia* and *Artemisia* they have shown that volatile terpenes from the leaves may inhibit root-growth of *Cucumis* seedlings placed in a closed container with them (Muller, Muller, and Haines, 1964). They have also demonstrated the presence of these terpenes in the air surrounding these shrubs in nature (Muller and Muller, 1964; Muller, 1965) and have postulated how they may enter the cells of victimized seedlings through solution in cuticular lipids (Muller, 1965).

The simplicity of the experimental set-up used by these workers in their demonstration of root-growth inhibition by volatile substances from shredded *Salvia* leaves suggested that this might form a suitable class exercise in an ecology course. Consequently, it was tried with the Evolutionary Plant Ecology course at the University of California, Berkeley, in the spring semester of 1964, with subsequent additional experiments which have been facilitated by National Science Foundation Research Grant No. G 21821. I am indebted to Charles Quibell who carried out the extra experiments, meantime making very valuable suggestions regarding technique.

Results obtained by the class were variable until the experimental design was fully standardized. The chambers and contents (fig. 1) were modeled after the setup described in Muller, Muller and Haines (1964). The seeds of *Cucumis sativus* L. were soaked in distilled water for 2 hours and then placed in position on the moist filter paper in the chambers. The glass lid to each chamber was fixed in place with petrolatum. The chambers were then kept together in the dark at 26.7°C (80°F) usually for 48 hours before measurements of root-growth were made. Only main roots were measured, laterals being ignored. The very few ungerminated seeds were also left out of account.

The availability of a small introduced population of *Salvia apiana* Jeps. on Grizzly Peak, Berkeley, enabled the results in Table 1A to be obtained. The differences between controls and test growths are all significant at the 1% level, confirming the findings of Muller et al.

However, it seemed likely that some other, more abundant local source of exocrines could be found. In the vicinity of Berkeley there are numerous plantations of *Eucalyptus globulus* Labill. under which very few

TABLE 1. VOLATILE GROWTH INHIBITORS PRODUCED BY EUCALYPTUS GLOBULUS

		Duration of Test (hours)	No. of Seedlings	Mean root- length (cms.)	Standard devia- tion	% of growth of control
A. <i>Salvia</i> leaves — <i>Cucumis</i> roots						
Control	(3 chambers)	48	142	2.20	0.56	
Salvia 1			42	1.62	0.54	74
Salvia 2			48	1.47	0.48	67
Salvia 3			42	1.48	0.48	67
Control	(2 chambers)	48	90	1.93	0.64	
Salvia	(2 chambers)		94	1.31	0.53	68
Control	(2 chambers)	60	83	2.86	1.04	
Salvia	(2 chambers)		70	2.09	0.60	73
Control	(4 chambers)	70	185	3.04	0.95	
Salvia	(3 chambers)		126	1.23	0.48	41
B. <i>Eucalyptus</i> leaves — <i>Cucumis</i> roots						
Control	(3 chambers)	48	142	2.20	0.56	
Eucalyptus 1			46	1.68	0.57	76
Eucalyptus 2			48	1.34	0.46	61
Eucalyptus 3			44	1.61	0.56	73
Control	(2 chambers)	48	90	1.93	0.64	
Eucalyptus	(2 chambers)		73	0.38	0.24	20
Control	(2 chambers)	60	83	2.86	1.04	
Eucalyptus	(2 chambers)		73	0.71	0.35	25
C. <i>Eucalyptus</i> leaves — <i>Eucalyptus</i> roots						
Control	(a)	24	50	0.67	0.27	
Juvenile Eucal.	(a)		51	0.56	0.17	83
Mature Eucal.	(a)		50	0.57	0.16	85
Control	(b)	24	48	0.91	0.11	
Juvenile Eucal.	(b)		50	0.83	0.25	91
Mature Eucal.	(b)		50	1.06	0.32	117
Control	(c)	24	10	1.35	0.32	
Juvenile Eucal.	(c)		10	1.33	0.30	99
Mature Eucal.	(c)		12	1.25	0.37	93
D. <i>Eucalyptus</i> leaves — <i>Eucalyptus</i> hypocotyls						
Control	(a)	24	50	0.33	0.07	
Juvenile Eucal.	(a)		51	0.25	0.05	76
Mature Eucal.	(a)		50	0.21	0.04	64
Control	(b)	24	48	0.43	0.15	
Juvenile Eucal.	(b)		50	0.28	0.06	66
Mature Eucal.	(b)		50	0.27	0.06	62
Control	(c)	24	10	0.48	0.14	
Juvenile Eucal.	(c)		10	0.25	0.04	53
Mature Eucal.	(c)		12	0.31	0.05	68

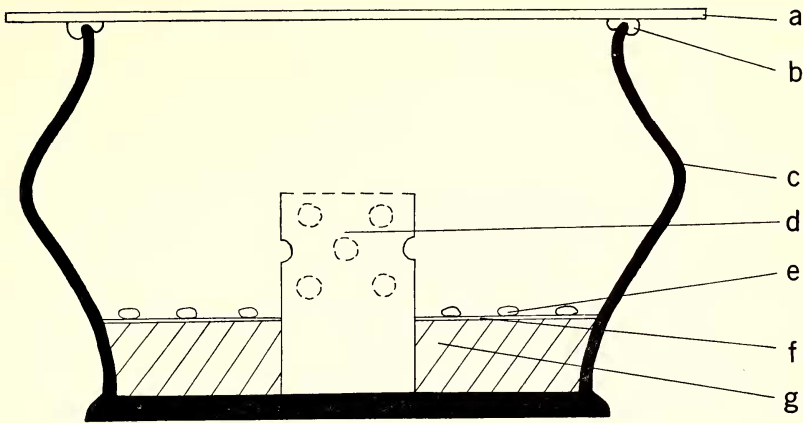


FIG. 1. Diagrammatic section through test chamber: a, glass lid; b, petrolatum seal; c, chamber; d, perforated container for test leaves; e, test seeds; f, filter paper; g, sponge, moistened with distilled water.

other plants grow. Although this forest tree has many natural associates in its native Australia, the contemporary California flora (a mixture of native species and mostly European introductions) appears generally to be unadapted to it as yet. The leaves of *E. globulus* are intensely aromatic, releasing a number of terpenes, including cineole (eucalyptole) (Baker and Smith, 1902). Cineole has been shown to be strongly inhibitory to root-growth (Muller and Muller, 1964) and to be one of the volatile toxins released by species of *Salvia* (Muller, 1965). Consequently, it seemed likely that terpenes from *E. globulus* might be responsible for the paucity of accompanying vegetation in the plantations.

Shredded *Eucalyptus* leaves proved to be just as efficacious as *Salvia* leaves in the inhibition of *Cucumis* seedling root-growth in class experiments in May, 1964 and in subsequent experiments in July 1964 (table 1B).

If volatile exoclines from *Eucalyptus* trees can be inhibitory to the root-growth of seedlings of other species, it is of interest to know their effects on the growth of their own seedlings. A modification of the experimental procedure was necessary in testing this, however, because *Eucalyptus* seed does not have the "simultaneous" germination pattern shown by *Cucumis*. It was necessary to germinate rather a large quantity of *Eucalyptus* seed after soaking for 48 hours in distilled water and then pick out seedlings with radicles of comparable length with which to begin the tests in the chambers. Table 1C shows the results obtained with shredded juvenile and adult leaves of *E. globulus* in the test chambers (the three sets of experiments being begun with germinated *Eucalyptus* seed having radicles of approximately (a) 1 mm, (b) 2 mm and (c) 3 mm, respectively).

Although exact comparisons between experiments carried out at separate times are not completely valid (and amounts of leaf were not weighed exactly), it seems that the leaves tested in July 1964 were more effective in inhibiting root-growth than those sampled earlier in the year, in May. This might reflect a difference in terpene production at these times.

For *E. globulus* root-growth, the test-results vary around 100% and none is significantly different at the 1% level from the controls, suggesting that the roots of this species are little affected by the volatile substances produced by the leaves of the same species. This might be advantageous in allowing more than one plant of the species to establish itself from seed in a newly-invaded area. On the other hand, there was a consistent effect ( $P \leq 0.01$ ) on the elongation of the hypocotyls of the *Eucalyptus* seedlings (table 1D). Several other experiments than those reported gave similar results with hypocotyl elongation. The ecological import of this finding has yet to be estimated.

The lack of effect of the volatile exocrines from *Eucalyptus* on the root-growth of its own seedlings recalls the apparent lack of sensitivity of the cells of *Colchicum* plants to the polyploidizing action of colchicine and the insensitivity to mutagenic allyl compounds shown by plants which produce them.

That plants will not always prove to be insensitive to the chemical products of their own species, however, is suggested by the observations of Went (1955) on creosote bushes (*Larrea divaricata* Cav.) which are often spaced remarkably evenly on the desert floors in California. Went attributes this to the susceptibility of *Larrea* seedlings to exocrines produced from the roots of existing creosote bushes, as a result of which seedlings only become established outside the radius of the circle of roots of existing shrubs.

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